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Norepinephrine augmented *in vitro* growth of uropathogenic *E. coli* in Type 2 diabetes mellitus and its suppression by silodosin (alpha blocker)☆,☆☆

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ABSTRACT

Norepinephrine is secreted under conditions of stress in humans. The ability of bacteria to sense mammalian hormone may have a role in propagation of infection. The present study investigated the effect of norepinephrine on *in vitro* growth of uropathogenic *E. coli* (UPEC) and the effect of silodosin on norepinephrine-induced changes. The spot urine samples were collected from 56 individuals (14 diabetic patients with UTI, 14 diabetic without UTI, 14 non-diabetic UTI and 14 healthy volunteer controls) for the measurement of urinary norepinephrine concentrations. The concentration of norepinephrine, as found in urine of human subjects, was reproduced in artificial urine medium to study the growth of UPEC. The norepinephrine concentration showing maximum growth response was selected to study the effect of silodosin on the growth inhibition of UPEC. Result showed significantly elevated urinary norepinephrine in diabetic patients with and without UTI and also in nondiabetic UTI groups. The norepinephrine concentration equivalent to that in diabetic UTI patients enhanced the growth of UPEC. Furthermore, silodosin (0.32 μM) inhibited the growth of the UPEC.

1. Introduction

Diabetes mellitus (DM) is the most common endocrine disorder that affects more than 415 million people all over the world; India (76.04 million) has second largest number of diabetic patients in the world (International Diabetes Federation, 2015). Diabetes mellitus is associated with a higher risk of infections. The urinary tract infection (UTI) is the most common infection in diabetes. The prevalence of UTI and its recurrence is 3 to 4 times higher in diabetic patients in comparison to nondiabetics (Fu et al., 2014; Geerlings, 2008; Hirji et al., 2012). In addition, the complications of UTI such as emphysematous cystitis, pyelonephritis, renal or perinephric abscess, bacteremia, and renal papillary necrosis are also more common in diabetic patients (Griffin et al., 1995; Huang and Tseng, 2000; Mnif et al., 2013). Immune dysfunction, glycosuria, voiding dysfunction and urinary retention, generally found in diabetes, may increase the susceptibility to UTI (Delamaire et al., 1997; Geerlings and Hoepelman, 1999; Geerlings et al., 1999; Hosking et al., 1978; Truzzi et al., 2008). However, the exact mechanism for higher risk of UTI in diabetes is not well understood.

Many studies have described an association between stress and susceptibility towards infection. Stress hormone catecholamines (epinephrine and norepinephrine) modulate immunological defense against infection and induce growth in various gram-negative and gram-positive bacteria (Belay et al., 2003; Lyte and Ernst, 1992; Peterson et al., 1991). In addition, norepinephrine (NE) can also influence production of virulence factors such as toxins and adhesions, biofilm production of various gut related bacteria (Bansal et al., 2007; Dowd, 2007; Karavolos et al., 2008, 2011; Lyte et al., 1997; Sandrini et al., 2015). Studies have also documented that mammalian α adrenergic receptor blockers suppress NE-induced growth and virulence factors (Freestone et al., 2007). Diabetes has life changing consequences for affected individuals, as evidenced by higher rates of anxiety and depression (Anderson et al., 2001; Collins et al., 2009). Studies have reported that people with anxiety and depression have elevated urinary NE (Grossman and Potter, 1999; Hughes et al., 2004). The findings suggest higher probability of urinary NE in diabetic patients and it may play a role in progression of UTI in diabetic population. The objectives of this study were to measure urinary NE concentrations in UTI patients, to study the *in vitro* growth pattern of uropathogenic *E. coli* (UPEC) in the presence of these pathological concentrations of NE and to investigate the activity of silodosin (α adrenergic receptor blockers) on the NE-induced effect.

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2. Material and methods

2.1. Patient selection

A total of 56 individuals (age between 35 and 55 years) were enrolled in the study and categorized into following 4 groups (Fig. 1), Group I included 14 type 2 diabetes patients with UTI (D-UTI), Group II included 14 type 2 diabetes patients without UTI (DC), Group III included 14 non-diabetic UTI patients (ND-UTI) and Group IV included 14 nondiabetics without UTI healthy volunteers as controls (HC).

Out-patients and in-patients of the Sir Sundar Lal Hospital, Banaras Hindu University, Varanasi, India, were screened for enrolment. The type 2 diabetes and UTI were diagnosed according to guidelines of American Diabetes Association, and European Association of Urology respectively (American Diabetes Association, 2014; EAU (European Association of Urology), n.d.). The study was approved by Institutional Ethical Committee, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. All subjects provided written informed consent.

2.2. Urine sampling and estimation of NE concentrations

The random spot urine samples were collected from individuals and acidified with 6 N HCl to maintain the pH below 3.0. Acidified urine was stored at −80 °C for the estimation of NE concentrations. Solid phase enzyme-linked immunosorbent assay (ELISA) kits (IBL International Hamburg, Germany), based on the sandwich principle, was used to quantify NE in the samples. The experiment was performed as per the manufacturer's instructions. The standard curve was used to derive the NE concentration for each unknown sample and expressed in μM.

2.3. Bacterial strain and inoculum preparation

The bacterial strain ($n = 1$) used in the study was uropathogenic *E. coli* (UPEC), which was isolated earlier from a diabetic UTI patient. The isolated bacterium was identified using biochemical and molecular methods as described previously (Clermont et al., 2000; Forbes et al., 2002; Padmavathy et al., 2012).

The bacterial strain was cultured overnight in 10 ml Luria Bertani (LB) medium at 37 °C 1 day prior to the experiment. The cultured bacteria was harvested and washed thrice with phosphate buffer saline (PBS) by centrifuging at 3000 g for 10 min. Bacterial pellets were resuspended in 10 ml PBS (pH 7.4). The desired inoculum (10^3 CFU/ml) was prepared by 10 fold serial dilution, which was confirmed by the standard pour plate method.

2.4. Preparation of medium

All the experiments were performed in artificial urine medium (AUM) having pH 7 which supports the growth of uropathogens, thereby providing similar physiological conditions as in urine. AUM was prepared as by the established protocol of Brooks and Keevil (1997).

2.5. Norepinephrine and its antagonist (silodosin)

Norepinephrine (bitartrate salt) and silodosin (α-adrenoreceptor antagonists) were purchased from Sigma-Aldrich, St. Louis and Cayman Chemicals, USA, respectively. The NE stock solution (10^{-4} M) was prepared in 0.1 N HCl and stock of silodosin (10^{-3} M) was prepared in DMSO. Both the stock solutions were sterilized using 0.2 Am syringe-tip filter.

2.6. Bacterial growth assay

The bacterial growth assay was performed in the AUM, supplemented with various NE concentrations (mean) identified in our study groups. For each NE concentration, a separate study was conducted. The media supplemented with NE was inoculated at a concentration of 10^3CFU mL^{-1} from the prepared bacterial inoculum. Inoculated media without NE was used as a control. The media with

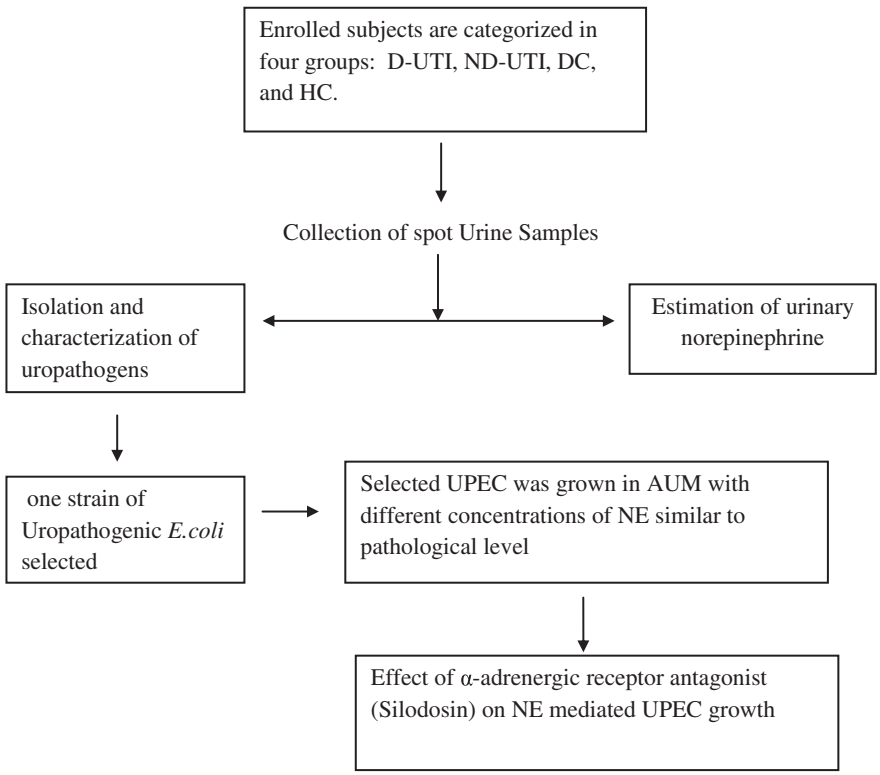

Fig. 1. Flow chart of study design.

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